



First you add knowledge...

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February 28, 2006

Ms. Felicia Billingslea, Director
Food Labeling and Standards (HFS 820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

**RE: Notification Submitted Pursuant to Section 403(w)(7) of the
Food Allergen Labeling and Consumer Protection Act**

Dear Ms. Billingslea:

Pursuant to Section 403(w)(7)(A)(i) of the Federal Food, Drug and Cosmetic Act, Danisco USA Inc. ("Danisco") respectfully submits the attached notification requesting an exemption from the labeling requirements of subsection 403(w)(1) set forth by the Food Allergen Labeling and Consumer Protection Act (FALCPA) for Danisco's anhydrous lactitol and lactitol monohydrate.

Danisco's lactitol products are produced by the catalytic hydrogenation of lactose (milk sugar), but do not contain allergenic protein, as demonstrated by the attached scientific documentation.

Four copies of this notice are enclosed herein for your review. We respectfully request your concurrence with our conclusion that Danisco's anhydrous lactitol and lactitol monohydrate are exempt from allergen labeling under FALCPA.

Please do not hesitate to contact me if you need additional information. We look forward to hearing from you soon.

Sincerely,

A handwritten signature in black ink that reads 'Michael H. Auerbach'. The signature is fluid and cursive, with the first and last names being more prominent.

M. H. Auerbach

**Notification Submitted Pursuant to Section 403(w)(7) of the
Food Allergen Labeling and Consumer Protection Act**

Anhydrous Lactitol and Lactitol Monohydrate

Submitted By: **Danisco USA Inc.**
 440 Saw Mill River Road
 Ardsley, NY 10502

February 28, 2006

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Notification Submitted Pursuant To Section 403(w)(7) Of The Food Allergen Labeling and Consumer Protection Act

Danisco USA Inc. hereby provides notification to the US Food and Drug Administration, pursuant to Section 403(w)(7) of the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Allergen Labeling and Consumer Protection Act (FALCPA), that Danisco's anhydrous lactitol and lactitol monohydrate products do not contain allergenic protein derived from milk and are exempt from allergen labeling.

As will be discussed in more detail below, FALCPA establishes a notification process for obtaining an exemption from the definition of "major allergen" in those instances when there are data demonstrating that an ingredient derived from a major allergen does not contain "allergenic protein." The basis for this notification is that anhydrous lactitol and lactitol monohydrate, while derived from lactose containing small amounts of residual milk protein do not contain "allergenic protein" derived from milk and hence should be exempt from allergen labeling.

I. Legal Basis for Filing a FALCPA Notification

A. Statutory Background

FALCPA establishes labeling requirements for major allergens. FALCPA amended the FFDCA and deems a food misbranded "if it is, or it contains an ingredient that bears or contains, a major food allergen" unless the food is labeled in accordance with one of two labeling options (1). A "major food allergen" is defined in paragraph (1) of subsection 201(qq) of the FFDCA as milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans, collectively known as the "Big 8 Allergens." A major food allergen also includes "a food ingredient that contains protein derived from a food specified in paragraph (1)," with the exception of highly refined oils and food ingredients that are exempt under the petition and notification procedures found in the statute (2).

FALCPA establishes the requirements for determining whether an exemption can be achieved through a notification or petition process (3). A notification is appropriate when there is "(i) scientific evidence (including the analytical method used) that demonstrates that the food ingredient (as derived by the method specified in the notification, where applicable) does not contain allergenic protein; or (ii) a determination by [FDA] that the ingredient does not cause an allergic response that poses a risk to human health under a premarket approval or notification program under section 409" (4). In instances when an ingredient does not qualify for the notification process, a petition can be filed requesting FDA to exempt a food ingredient from the labeling requirements by providing "scientific evidence...that demonstrates that such food ingredient...does not cause an allergic response that poses a risk to human health." (5)

B. Justification for a FALCPA Labeling Exemption

As indicated above, it is appropriate to submit a notification to exempt anhydrous lactitol

and lactitol monohydrate from the allergen labeling requirements of FALCPA because scientific evidence demonstrates that these products do not contain any detectable allergenic protein. FALCPA requires the notification to contain the scientific evidence, including the analytical method, demonstrating that the food ingredient produced by the method described in the notification does not contain allergenic protein.

This Notification satisfies these requirements and contains the following information:

- (1) a description of lactitol,
- (2) the method used to manufacture lactitol,
- (3) scientific data demonstrating that the lactitol products do not contain allergenic protein, and
- (4) the analytical methods used to demonstrate that the lactitol products do not contain allergenic protein.

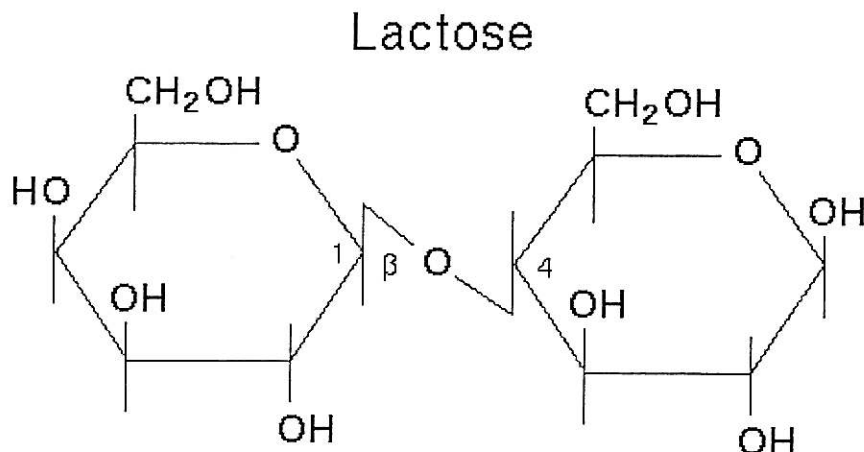
II. Lack of Allergenic Protein in Danisco Lactitol Products

The notification process is appropriate when there is scientific evidence establishing that the food ingredient does not contain allergenic protein. This evidence is detailed below.

A. Description of Lactitol Products

Lactitol is a sugar alcohol (polyol). It has a mild sweet taste and provides 2.0-2.4 kcal/g energy content vs. 4 kcal/g for most sugars and carbohydrates. Lactitol is used as an ingredient for specialty sweetening applications in the food, pharmaceutical and health care industries.

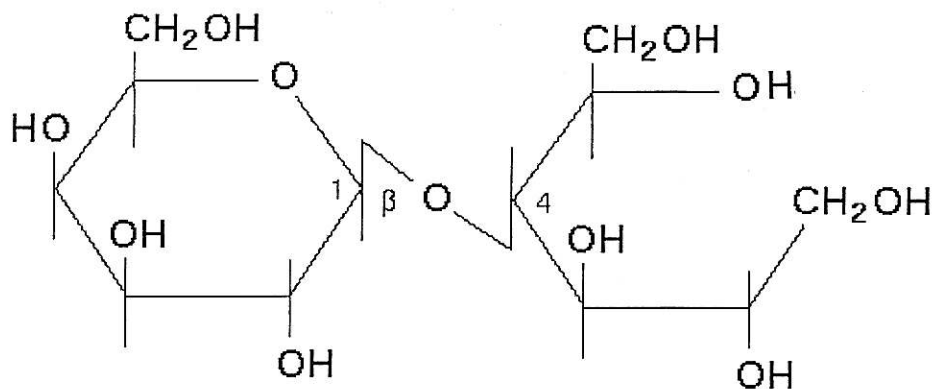
Lactitol is produced from lactose (milk sugar). Lactose is the principal carbohydrate in milk, comprising 2-8% of the milk solids. It is a disaccharide, consisting of β -D-galactose and α -D-glucose molecules bonded through a β 1-4 glycosidic linkage (see below). Commercial lactose contains up to 0.2% (2000 ppm) total protein by specification.



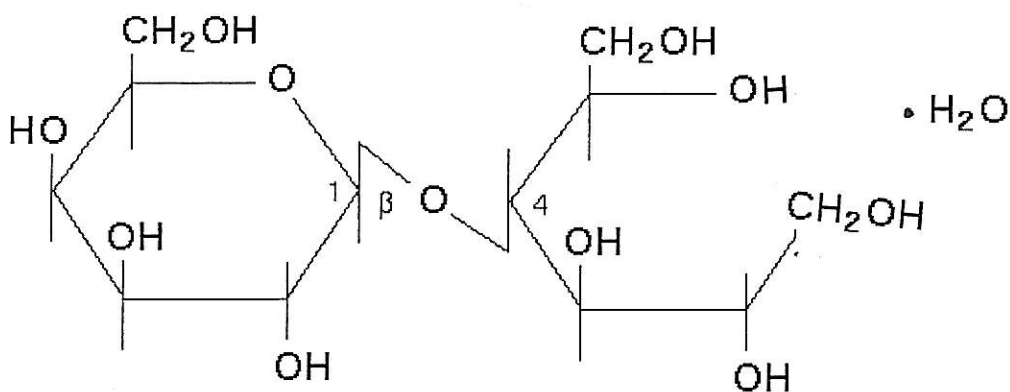
<u>Product</u>	<u>Formula</u>	<u>Mol. wt.</u>	<u>CAS RN</u>
Anhydrous lactitol	$C_{12}H_{24}O_{11}$	344.31	585-86-4
Lactitol monohydrate	$C_{12}H_{24}O_{11} \cdot H_2O$	362.37	81025-04-9

The chemical structures of Danisco's lactitol products are shown below and technical specifications and representative certificates of analysis are given in Attachment 1.

Anhydrous lactitol



Lactitol monohydrate



B. Lactitol Manufacturing Process

An aqueous solution of lactose is catalytically hydrogenated over Raney nickel catalyst at temperatures over 115 °C. This reduces the anomeric carbon of the glucose moiety yielding lactitol. This process is essentially identical to that used to produce sorbitol from glucose. Anhydrous lactitol and lactitol monohydrate are produced by the same process with slight variations in the crystallization process conditions.

C. Total Protein Content in Lactose

As noted above, the protein content in commercial lactose is $\leq 0.2\%$ (2000 ppm) by specification.

D. Total Protein Content in Lactitol

The total protein content of duplicate samples of anhydrous lactitol and lactitol monohydrate was determined by Microbe Inotech Laboratories, St. Louis, MO, using the Lowry-Folin method (Attachment 2). The analytical report is given in Attachment 3. Microbe Inotech also applied the Bradford method (Attachment 4) to the same samples. The report is given in Attachment 5. The results are summarized below.

<u>Product</u>	<u>Sample</u>	<u>Lowry-Folin Method</u>	<u>Bradford Method</u>
Anhydrous Lactitol	N120T5C09A	416 ppm	570 ppm
Anhydrous Lactitol	N120T5L22	412 ppm	325 ppm
Lactitol Monohydrate	L120T5J06	487 ppm	258 ppm
Lactitol Monohydrate	L120T5M18	542 ppm	542 ppm

These values – 260-570 ppm - represent 13-28% of the original protein specification in the starting lactose. This is not allergenic protein according to further testing described below.

E. Allergenic protein in Lactitol

The milk allergen content of the same four samples above was determined at Medallion Laboratories, Minneapolis, MN, using the Neogen ELISA test for milk allergens (Attachment 6). The results are summarized below. The full report is given in Attachment 6.

<u>Product</u>	<u>Sample</u>	<u>Dairy Allergen Content</u>
Anhydrous Lactitol	N120T5C09A	< 2.5 ppm
Anhydrous Lactitol	N120T5L22	< 2.5 ppm
Lactitol Monohydrate	L120T5J06	< 2.5 ppm
Lactitol Monohydrate	L120T5M18	< 2.5 ppm

The limit of detection of this analysis is 2.5 ppm. Therefore the four lactitol samples contained no detectable milk allergens.

The Danisco Thomson, IL, manufacturing facility produces lactitol, xylitol, xylose and rhamnose. Aside from the lactose raw material purchased for the lactitol process, none of the materials used at the Thomson plant contains any of the major food allergens regulated under FALCPA. Furthermore, the lactitol process is essentially completely closed except for packaging, so there is no possibility of cross-contamination from any of the other major food allergens regulated under FALCPA.

III. Conclusion

Because there were no detectable milk allergens in any of Danisco's lactitol samples, and no possibility of cross-contamination from other major food allergens at the Thomson lactitol production facility, we submit that Danisco's lactitol products do not contain any major food allergens.

Accordingly, we respectfully request that FDA confirm that Danisco's anhydrous lactitol and lactitol monohydrate are exempt from allergen labeling under FALCPA.

IV. References

1. FFDCA § 403(w)(1).
 - One option involves placing the word "contains," followed by the name of the food source from which the major allergen is derived, either immediately after or adjacent to the ingredient statement.
 - The other option involves including the name of the food source from which the major allergen is derived in parentheses following the listing of the ingredient in the ingredient statement, except that such parenthetical listing is not necessary when the name of the food source appears as part of the listing of another major allergen in the food product.
2. See FFDCA § 201(qq)(2).
3. See FFDCA § 403(w)(6) and (7).
4. See FFDCA § 403(w)(7)(A)
5. See FFDCA § 403(w)(6)(C).

V. Attachments

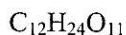
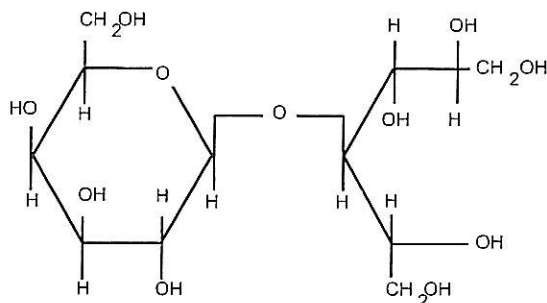
1. Technical Specifications and Certificates of Analysis
2. Bradford (Coomassie) Brilliant Blue Method for Determination of Total Protein Content
3. Results of Microbe Inotech Bradford Total Protein Analyses
4. Lowry-Folin Method for Determination of Total Protein Content
5. Results of Microbe Inotech Lowry Total Protein Analyses
6. - Neogen ELISA Method for Determination of Dairy Allergen Content
 - Results of ELISA determination of dairy allergen content of Danisco lactitol samples.

DANISCO

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TECHNICAL SPECIFICATION**LACTITOL AC**

Lactitol Anhydrous, Crystalline, Food Grade



Mol. Wt.: 344.37

DESCRIPTION:	Odourless, white crystalline powder with a mild, sweet taste.		
SOLUBILITY:	Soluble in water (approximately 1.9 g/ml @ 25°C).		
SPECIFICATIONS:	LIMITS:	METHOD:	
Colour (10% w/v solution)	Max. 40 ICUMSA	ICUMSA	
pH (10% w/v solution)	4.5 - 7.0	pH meter	
Lactitol (on dry substance)	98.0% – 102.0%	HPLC	
Other polyols (on d.s.)	Max. 1.5%	HPLC	
Reducing sugars (as lactose on d.s.)	Max. 0.2%	Luff Schoorl	
Melting range	147°C - 154°C	European Pharmacopoeia	
Moisture	Max. 0.5%	Karl Fischer	
Sulphated Ash	Max. 0.1%	European Pharmacopoeia	
Arsenic (on d.s.)	Max. 0.5 mg/kg	AAS – Hydride Method	
Heavy metals (on d.s.)	Max. 5 mg/kg	European Pharmacopoeia	
Lead (on d.s.)	Max. 0.15 mg/kg	ICP	
Nickel (on d.s.)	Max. 1 mg/kg	ICP	
Chloride (on d.s.)	Max. 30 mg/kg	USP	
Sulphate (on d.s.)	Max. 30 mg/kg	USP	

STABILITY IN STORAGE: LACTITOL AC is acceptably stable to air and heat and is only marginally hygroscopic. Goods in the original sealed packages at temperatures below 25°C and relative humidity less than 55% can be expected to retain stability for at least two years.

The information contained herein is based on data available to us and is believed to be accurate. However, no warranty is expressed or implied regarding the accuracy of this data or the results to be obtained from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. Freedom from patent restrictions should not be assumed.

August 19, 2003

Danisco USA Inc.
10994 Three Mile Road
61285 Thomson
Tlf. 815-259-3311
Fax 815-259-3153

DANISCO

Certificate of Analysis

Lactitol Anhydrous Cryst. 20kg bag (T)

Material 134576
Lot number N120T6B02
Old product number N120T

Page 1 of 1

Characteristic	Lower Limit	Upper Limit	Value	Unit
ICUMSA Color (NMT 40)	-	40	15	ICUMSA
pH	4.5	7.0	5.0	pH
Assay	98.0	102.0	99.1	%
Related Compounds	-	1.5	1.0	%
Reducing Sugars (As Dextrose)	-	0.2	0.0	%
Melting Range Begin	147	-	151	°C
Melting Range End	-	154	152	°C
Moisture (KF)	-	0.5	0.1	%
Residue on Ignition	-	0.10	0.00	%
Arsenic (NMT 0.5 PPM)	-	-	Passes Test	
Heavy Metals as Pb(NMT 5 PPM)	-	-	Passes Test	
Lead (NMT 0.15 PPM)	-	-	Passes Test	
Nickel (NMT 1 PPM)	-	-	Passes Test	
Chloride (NMT 30 PPM)	-	-	Passes Test	
Sulfate (NMT 30 PPM)	-	-	Passes Test	

Best Before Date:

Analysis result "Passes Test" means that the parameter is subject to reduced testing based on statistical data.

This material has been evaluated by Quality Control and is released for use. This certificate is electronically generated and has no signature.

Joseph Dixon
Quality Control Manager

02/06/2006

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US PRODUCT DESCRIPTION

Lactitol AC (Anhydrous, Crystalline)

Description

Lactitol is a sugar alcohol derived from milk sugar.

Lactitol anhydrous is a white crystalline powder, has a mild sweet taste and is odorless.

Typical Application Areas

Sugar Free / Reduced sugar / Reduced calorie

- Chocolate and compound coatings
- Confections

Benefits

- 2.0 kcal/g
- Similar stability to sucrose
- Sugarlike solubility and viscosity
- 0.3-0.4 times as sweet as sucrose

Usage Levels

The following guidelines can be given:

Chocolate and compound coatings	10-50%
Confections	10-80%

Direction for Use

Use within a week to ten days after opening bag to prevent clumping.

Specifications

pH in aqueous solution (0.1 g/ml)	4.5-7.0
Lactitol*	98.0% - 102.0%
Other polyols*	Max. 1.5%
Reducing Sugars*	Max. 0.2%
Moisture	Max. 0.5%
Sulphated Ash	Max. 0.1%
Total Plate Count	Max. 100 col/g

Yeast	Max. 10 col/g
Mold	Max. 10 col/g
Coliforms	Negative
Salmonella	Negative
E.coli	Negative
Arsenic*	Max. 0.5 mg/kg
Heavy metals*	Max. 5 mg/kg
Lead*	Max. 0.15 mg/kg
Nickel*	Max 1 mg/kg

*Solids basis

Nutrition Data

(Approximate values for nutrition labeling per 100 g)

Energy	200 kcal
Protein	None
Carbohydrate	100 g
- of which sugars	None
Fat	None
- of which saturated	None
Fiber	None
Sodium	1 mg

Ingredient Declaration

Lactitol

Transportation and Storage

Stable for at least two years in the original sealed packaging when stored at temperatures below 25°C at relative humidity less than 55%.

Packaging

- 20 kg multi wall paper bag

Regulatory and Legal Status

- USA – Self GRAS, USP/NF
- Europe – EP, E966
- Kosher certified
- Local food regulations should always be consulted concerning the status of this product, as legislation regarding its use in food may vary from country to country. Advice regarding the legal status of this product may be obtained on request.

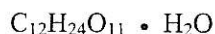
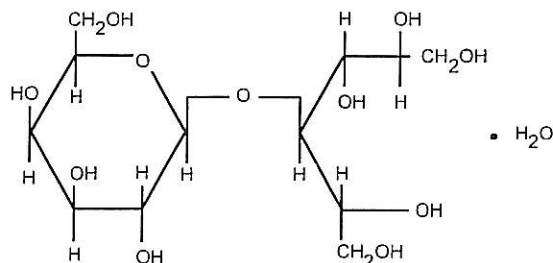
Purity/Safety and Handling

- The product is manufactured under a quality management system certified as compliant with the requirements of ISO 9002:1994.
- Material Safety Data Sheet available on request.

TECHNICAL SPECIFICATION

LACTITOL MC

Lactitol Monohydrate, Crystalline, Food Grade



Mol. Wt.: 362.37

DESCRIPTION: Odourless, white crystalline powder with a mild, sweet taste.

SOLUBILITY: Soluble in water (approximately 1.7 g/ml @ 25°C).

SPECIFICATIONS:

LIMITS:

METHOD:

Colour (10% w/v solution)	Max. 30 ICUMSA	ICUMSA
pH (10% w/v solution)	4.5 - 7.0	pH meter
Lactitol (on dry substance)	98.0% - 101.0%	HPLC
Other polyols (on d.s.)	Max. 1.5%	HPLC
Reducing sugars (as lactose on d.s.)	Max. 0.2%	Luff Schoorl
Moisture	Max. 4.5% - 5.5%	Karl Fischer
Sulphated Ash	Max. 0.1%	European Pharmacopoeia
Arsenic (on d.s.)	Max. 0.5 mg/kg	AAS - Hydride Method
Heavy metals (on d.s.)	Max. 5 mg/kg	European Pharmacopoeia
Lead (on d.s.)	Max. 0.15 mg/kg	ICP
Nickel (on d.s.)	Max. 1 mg/kg	ICP
Chloride (on d.s.)	Max. 30 mg/kg	USP
Sulphate (on d.s.)	Max. 30 mg/kg	USP

STABILITY IN STORAGE: LACTITOL MC is acceptably stable to air and heat and is only marginally hygroscopic. Goods in the original sealed packages at temperatures below 25°C and relative humidity less than 65% can be expected to retain stability for at least two years.

The information contained herein is based on data available to us and is believed to be accurate. However, no warranty is expressed or implied regarding the accuracy of this data or the results to be obtained from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. Freedom from patent restrictions should not be assumed.

September 5, 2003

Certificate of Analysis

Lactitol Monohydrate Cryst. 20kg bag (T)

Material 134517
Lot number L120T6A09
Old product number L120T

Page 1 of 1

Characteristic	Lower Limit	Upper Limit	Value	Unit
Color (NMT 30 ICUMSA)	-	-	Passes Test	
Lactitol pH 4.5 - 7.0	4.5	7.0	4.7	pH
Assay (db)	98.0	101.0	99.2	%
Related Compounds (db)	-	1.5	0.7	%
Reducing Sugars (As Dextrose)	-	0.2	0.0	%
Moisture (KF)	4.5	5.5	5.0	%
Residue on Ignition	-	0.1	0.0	%
Arsenic (NMT 0.5 PPM)	-	-	Passes Test	
Heavy Metals as Pb (NMT 5 PPM)	-	-	Passes Test	
Lead (NMT 0.15 PPM)	-	-	Passes Test	
Nickel (NMT 1 PPM)	-	-	Passes Test	
Chloride (NMT 30 PPM)	-	-	Passes Test	
Sulfates (NMT 30 PPM)	-	-	Passes Test	
Organic Volatile Impurities	-	-	Passes Test	

Best Before Date:

This material has been evaluated by Quality Control and is approved for use. The certificate of analysis is electronically generated and has no signature.

Joseph Dixon
Quality Control Manager

01/10/2006

11

US PRODUCT DESCRIPTION

Lactitol MC (Monohydrate, Crystalline)

Description

Lactitol is a sugar alcohol derived from milk sugar.

Lactitol monohydrate is a white crystalline powder, has a mild sweet taste and is odorless.

Typical Application Areas

Sugar Free / Reduced sugar / Reduced calorie

- Confections
- Ice cream
- Baked goods

Benefits

- 2.0 kcal/g
- Similar stability to sucrose
- Sugarlike solubility and viscosity
- 0.3-0.4 times as sweet as sucrose

Usage Levels

The following guidelines can be given:

Confections	10-80%
Baked Goods	5-15%
Ice Cream	5-10%

Direction for Use

No special handling required.

Specifications

pH in aqueous solution (0.1 g/ml)	4.5-7.0
Lactitol*	98.0% - 101.0%
Other polyols*	Max. 1.5%
Reducing Sugars*	Max. 0.2%
Moisture	Max. 4.5-5.5%
Sulphated Ash	Max. 0.1%
Total Plate Count	Max. 100 col/g
Yeast	Max. 10 col/g
Mold	Max. 10 col/g
Coliforms	Negative
Salmonella	Negative
E.coli	Negative
Arsenic*	Max. 0.5 mg/kg
Heavy metals*	Max. 5 mg/kg
Lead*	Max. 0.15 mg/kg
Nickel*	Max 1 mg/kg
Chloride*	Max. 30 mg/kg
Sulphate*	Max. 30 mg/kg

*Solids basis

Nutrition Data

(Approximate values for nutrition labeling per 100 g)

Energy	200 kcal
Protein	None
Carbohydrate	95 g
- of which sugars	None
Fat	None
- of which saturated	None
Fiber	None
Sodium	1 mg

Ingredient Declaration

Lactitol

Transportation and Storage

Stable for at least two years in the original sealed packaging when stored at temperatures below 25°C at relative humidity less than 65%.

Packaging

- 20 kg kraft bag

Regulatory and Legal Status

- USA – Self GRAS, USP/NF
- Europe – EP, E966
- Kosher certified
- Local food regulations should always be consulted concerning the status of this product, as legislation regarding its use in food may vary from country to country. Advice regarding the legal status of this product may be obtained on request.

Purity/Safety and Handling

- The product is manufactured under a quality management system certified as compliant with the requirements of ISO 9002:1994.
- Material Safety Data Sheet available on request.

TOTAL PROTEIN ASSAYS

- [Background](#)
 - [Bradford Assay Procedure](#)
 - [References](#)
 - [Return to Biochemistry 353](#)
-

Background & Theory

Four spectroscopic methods are routinely used to determine the concentration of protein in a solution (1). These include measurement of the protein's intrinsic UV absorbance and three methods which generate a protein-dependent color change; the Lowry assay (2), the Smith copper/bicinchoninic assay (3) and the Bradford dye assay (4). Although one or more these methods is used routinely in almost every biochemical laboratory, none of the procedures are particularly convenient, for the reasons described below.

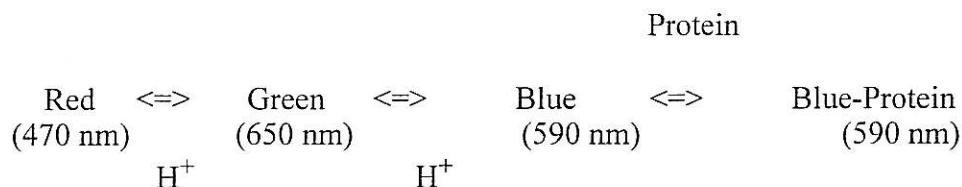
The first, UV absorbance, requires that a pure protein with known extinction coefficient be used, in a solution free of interfering (UV absorbing) substances. As an approximation, the protein concentration of a solution can be estimated by using either of the following equations;

$$A_{280} = 1 A (\text{mL/cm mg}) \times [\text{Conc.}] (\text{mg/mL}) \times 1 (\text{cm})$$
$$A_{205} = 31 A (\text{mL/cm mg}) \times [\text{Conc.}] (\text{mg/mL}) \times 1 (\text{cm})$$

Different proteins, however, have widely different extinction coefficients at both 280 and 205 nm, and concentration estimates obtained this way should be viewed with considerable skepticism. Again, this assay requires that the protein solution be free of other UV absorbing substances, and that the measurements be made using a quartz cuvette.

The Lowry and copper/bicinchoninic assays are based on reduction of Cu^{2+} to Cu^{1+} by amides. Although this makes them potentially quite accurate, they require the preparation of several reagent solutions, which must be carefully measured and mixed during the assay. This is followed by lengthy, precisely timed incubations at closely controlled, elevated temperatures, and then immediate absorbance measurements of the unstable solutions. Both assays may be affected by other substances frequently present in biochemical solutions, including detergents, lipids, buffers and reducing agents (1). This requires that the assays also include a series of standard solutions, each with a different, known concentration of protein, but otherwise having the same composition as the sample solutions.

The Bradford dye assay is based on the equilibrium between three forms of Coomassie Blue G dye. Under strongly acid conditions, the dye is most stable as a doubly-protonated red form. Upon binding to protein, however, it is most stable as an unprotonated, blue form.



The Bradford assay is faster, involves fewer mixing steps, does not require heating, and gives a more stable colorimetric response than the assays described above. Like the other assays, however, its response is prone to influence from non protein sources, particularly detergents, and becomes progressively more nonlinear at the high end of its useful protein concentration range. The response is also protein dependent, and varies with the composition of the protein. (See examples) These limitations make protein standard solutions necessary.

The reagents for all of the protein assays are widely available, and pre-weighed reagents (5), reagent mixtures (5), and reagent solutions (6,7) are available for the assays. Modifications of these assays, using proprietary solutions (8) or different protocols or formats are also described in the literature (9) or are commercially available (10).

BRADFORD PROTEIN ASSAY PROCEDURE

Reagents:

Dye stock - Coomassie Blue G (C.I.# 42655) (100 mg) is dissolved in 50 mL of methanol. (If turbid, the solution is treated with Norit (100 mg) and filtered through a glass-fiber filter.) The solution is added to 100 mL of 85% H_3PO_4 , and diluted to 200 mL with water. The solution should be dark red, and have a pH of -0.01. The final reagent concentrations are 0.5 mg/mL Coomassie Blue G, 25% methanol, and 42.5% H_3PO_4 . The solution is stable indefinitely in a dark bottle at 4°C.

Assay reagent - The assay reagent is prepared by diluting 1 volume of the dye stock with 4 volumes of distilled H_2O . The solution should appear brown, and have a pH of 1.1. It is stable for weeks in a dark bottle at 4°C.

Protein Standards - Protein standards should be prepared in the same buffer as the samples to be assayed. A convenient standard curve can be made using bovine serum albumin (BSA) with concentrations of 0, 250, 500, 1000, 1500, 2000 $\mu\text{g/mL}$ for the standard assay, and 0, 10, 20, 30, 40, 50 $\mu\text{g/mL}$ for the microassay.

Standard Protein Assay Procedure (200 - 2000 $\mu\text{g/mL}$ protein):

Prepare six standard solutions (1 mL each) containing 0, 250, 500, 1000, 1500 and 2000 $\mu\text{g/mL}$ BSA. Set the spectrophotometer to collect the spectra over a wavelength range from 400 to 700 nm and over an absorbance range of 0 to 2 Absorbance units, and to overlay the collected spectra. Use a 4 mL plastic cuvette filled with distilled water to blank the spectrophotometer over this wavelength range

Empty the plastic cuvette into a test tube and shake out any remaining liquid. Then add;

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2.0 mL Assay reagent

0.04 mL of protein standard solution, starting with the lowest protein concentration and working up, or one of the samples to be assayed.

Cover with Parafilm and gently invert several times to mix

Record the absorbance spectrum of the sample from 400 to 700 nm, and note the absorbance at 595 nm.

Repeat the steps above for each of the protein standards and for the samples to be assayed.

Examine the spectra of the standards and samples. If any spectrum has an absorbance at 595 nm greater than 2, or if any sample has an absorbance greater than the greatest absorbance for any of the standards, dilute the sample by a known amount and repeat the assay. At one wavelength, approximately 575 nm, all of the spectra should have the same absorbance. (Such an intersection is called an isosbestic point and is a defining characteristic of solutions containing the same total concentration of an absorbing species with two possible forms.) If any spectrum does not intersect the other spectra at or near the isosbestic point, it should be adjusted or rejected and repeated. (It is sometimes possible to adjust the baseline of a spectrum by determining the difference in absorbance at the isosbestic point from the absorbance of the other spectra at that wavelength, and adding the difference to the absorbance values at every wavelength of the spectrum. This correction works best at wavelengths close to the isosbestic point, and requires some discrimination by the spectroscopist.)

Prepare a graph of Absorbance at 595 nm vs [Protein] for the protein standards.

Examine the graphed points and decide if any should be rejected. (Often a single point can be rejected without invalidating the standard curve, but if more than one point appears questionable the assay should be repeated.) The Bradford assay gives a hyperbolic plot for absorbance versus protein concentration, but within a range of relatively low protein concentrations, the hyperbolic curve can be approximated reasonably well by a straight line. Use a best-fit straight line to fit the points if you feel it will give a good fit. If not, draw a smooth curve that falls on or near each of the data points.

To determine the protein concentration of a sample from its absorbance, use the standard curve to find the concentration of standard that would have the same absorbance as the sample.

Microassay Procedure (<50 µg/mL protein):

Prepare five standard solutions (1 mL each) containing 0, 10, 20, 30, 40 and 50 µg/mL BSA
To a 1.4 mL plastic cuvette, add;

0.2 mL Dye stock

0.8 mL of one of the protein standard solutions or samples to be assayed (containing <100 µg of protein for <50 µg/mL standards)

Cover with Parafilm and gently invert several times to mix.

Follow the procedure described above for the standard assay procedure.

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REFERENCES

1. Stoscheck, C. 1990 "Quantification of Protein" *Methods in Enzymology* , **182**:50-68.
2. Lowry, O. Rosebrough, A., Farr, A. and Randall, R. 1951 *J. Biol. Chem* . **193**:265.
3. Smith, P. et al., (1985) *Anal. Biochem.* **150**:76-85.
4. Bradford, M. 1976 "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" *Anal. Biochem.* **72**:248-254.
5. anon. "Sigma Modified Lowry Protein Assay," Sigma Procedure No. P 5656.
6. anon. 1991 "BCA Protein Assay Reagent," Pierce catalog p. F-18.
7. anon. 1991 "Bio-Rad Protein Assay," Bio-Rad catalog p. 60.
8. anon. 1991 "Coomassie Plus Protein Assay Reagent" Pierce catalog p. F-22.
9. Cabib, E. and Polacheck, I. 1984 "Protein assay for dilute solutions." *Methods in Enzymology*, **104**:318-328.
10. anon. 1991 "Fast Protein Assay," Pierce catalog F-26.

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[Return to Biochemistry 253a](#)

Use of the Bradford Protein Assay in a Microtiter Plate Format

Saban Tekin and Peter J. Hansen
Dept. of Animal Sciences, University of Florida

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University of Florida

• Introduction

The Bradford protein assay is a simple procedure for determination of protein concentrations in solutions that depends upon the change in absorbance in Coomassie Blue G-250 upon binding of protein (Bradford, *Anal. Biochem.* 72: 248, 1976). Unlike many other assays, including the Lowry procedure, the Bradford assay is not susceptible to interference by a wide variety of chemicals present in samples. The notable exception is high concentrations of detergents. There is significant protein-to-protein variation in absorbance values obtained with the Bradford procedure and it is advisable to choose a protein standard that is likely to give absorbance values close to those for the protein samples of interest.

The assay here is designed for use in microtiter plates. This is an easy assay format for those with access to multiple channel pipettors and microtiter plate spectrophotometers.

• Materials

Bradford Reagent - Bio-Rad sells a ready-to-use reagent (cat#500-0006) that can be stored at 4 C. Alternatively, the reagent can be made by dissolving 100 mg Coomassie Blue G-250 (available from several sources) in 50 ml 95% ethanol, adding 100 ml 85% (w/v) phosphoric acid to this solution and diluting the mixture to 1 liter with water.

Bovine serum albumin (BSA) (1 mg/ml) - we dissolve BSA in saline and store it frozen in 1 ml aliquots for quick use. The standard should be dissolved in a buffer similar to that the unknowns will be dissolved in. Note: other standards are acceptable and may be preferred for certain applications (for example, use an IgG standard when measuring concentrations of immunoglobulins).

Microtiter plates - we use Micro Test III Flexible assay plate from Falcon because they are cheap and work well.

Any multi-well microtiter plate reader equipped with a 595 nm filter.

• Procedure

Note: do all determinations in duplicate or triplicate

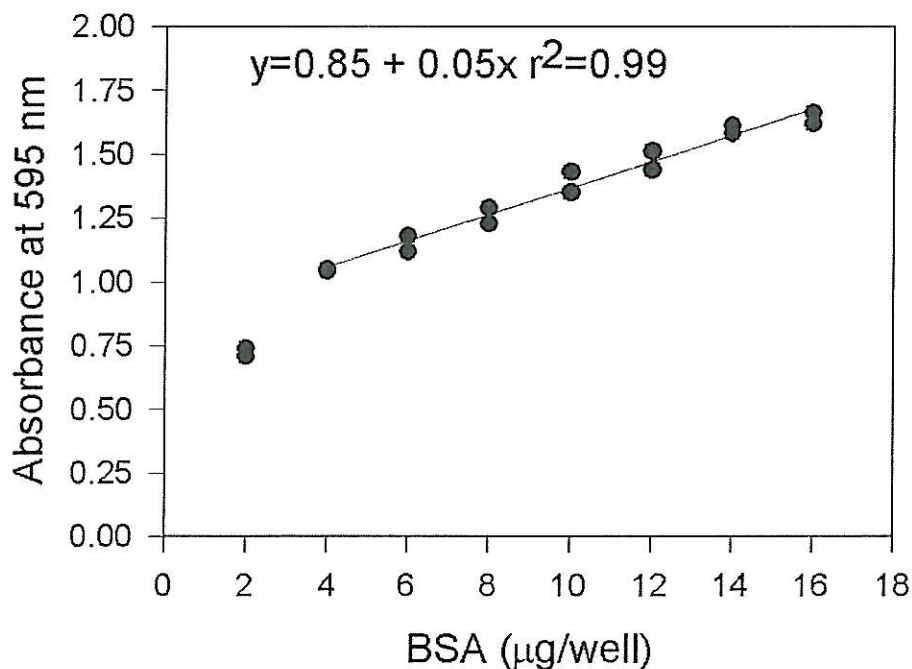
1. Pipette 0, 2, 4, 6, 10, 15 and 20 μ l of BSA (1 mg/ml) into assigned wells of a 96-well plate.
2. Pipette up to 20 μ l of unknown samples into individual wells of a 96-well plate.
3. Add 40 μ l of Bradford Reagent into all wells containing standard or sample.

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4. Add dd H₂O to all wells to bring the final volume to 200 µl.
5. Read absorbance at 595 nm without any prior incubation.

Representative Standard Curve

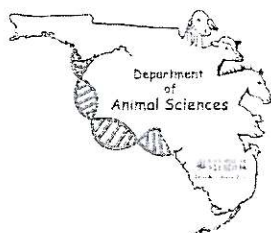
Note non-linearity at low end of the standard curve



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created 2-4-00 modified 3-9-01

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Department of Animal Sciences - Dairy Program

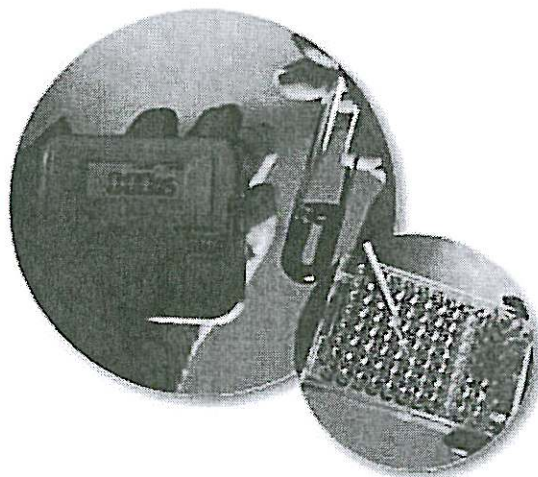
18

PIERCE



Coomassie Plus - The Better Bradford Assay

As fast as the original Coomassie Assay, with increased accuracy...the high performance Bradford reagent.



Choose the fastest and simplest protein assay reagent available! The Coomassie Plus Assay Reagent is ready to use – no tedious working reagent preparation is necessary. Simply add the reagent to the equal volumes of samples and standards, mix and then measure the absorbance at 595 nm. The assay costs only pennies per sample and may be performed in either test tube or microplate format. The Coomassie Plus Assay Reagent formulation provides increased linearity of response and only half the expected protein:protein variation of other commercial Bradford assay formulations.

Highlights:

- Detects protein concentrations from 1-1,500 $\mu\text{g/ml}$
- Ready-to-use dye-binding reagent formulation
- Fast (almost immediate) color development read at 595 nm
- Compatible with reducing sugars, reducing substances and thiols
- Refrigerated reagent is stable for 2 years
- Superior linear response over the range of 125-1,500 $\mu\text{g/ml}$
- Adaptable to microplates
- Micro protocol useful for protein concentrations from 1-25 $\mu\text{g/ml}$

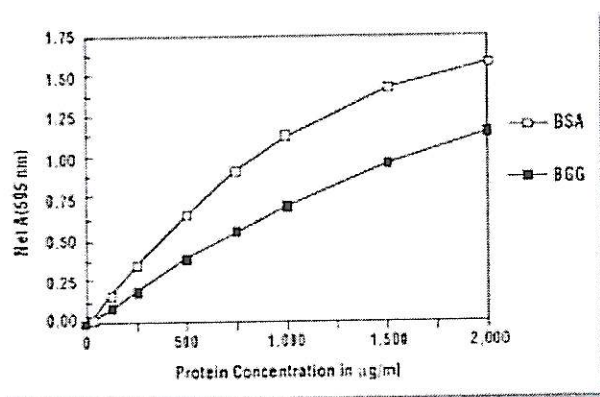


Figure 1. Typical color response curves for BSA and BGG using the Coomassie Plus - The Better Bradford Assay Reagent.

Compatible Substances

Reagents compatible with Coomassie Plus - The Better Bradford Assay Kit using the

standard protocol. Interferences may be observed at the stated concentration when using the Micro Assay Procedure.

Ammonium Sulfate	1.0 M	β -Mercaptoethanol	1.0 M
Azide	0.5%	MES	100 mM
NaCl	5.0 M	NaOH	0.1 M
Brij-35	0.062%	Brij-56	0.03%
Brij-58	0.016%	NP-40	0.5%
CHAPS	5.0%	CHAPSO	5.0%
SDS	0.016%	Sucrose	10.0%
Citrate	200 mM	Tris	2.0 M
EDTA	0.1 M	Glucose	1.0 M
Glycine	0.1 M	Triton X-100	0.062%
Triton X-114	0.062%	Triton X-405	0.25%
Guanidine HCl	3.5 M	Tween-20	0.031%
Tween-80	0.016%	HCl	0.1 M
KSCN	3.0 M	Urea	3.0 M

- **Easier, Quicker Preparation**

Working reagent is ready to use. No tedious dilution, no filtration of a dye concentrate and no mess to clean up.

- **Lower Cost Per Assay**

Just 19¢ per sample with the standard protocol, 12¢ per sample with the micro protocol and 4¢ per sample with the microplate protocol

- **Faster Assay**

Total assay time is less than five minutes

- **More Accurate Results**

Substantially increased linearity of response, and only half the expected protein-to-protein variation of other commercial formulations.

References:

1. Bradford, M. (1976). *Anal. Biochem.* **72**, 248-254.
2. Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
3. Kagan, A., et al. (2000). *J. Biol. Chem.* **275**, 11241-11248.
4. Goel, R., et al. (2002). *J. Biol. Chem.* **277**, 18640-18648.

Images

[Chart Image for Product Family Coomassie Plus - The Better Bradford Assay Kit](#)

[Graph Image for Product Family Coomassie Plus - The Better Bradford Assay Kit](#)

Ordering Information



Certificate of Analysis



Instruction Book with Protocols



MSDS

Buy	Product #	Description	Pkg. Size	Files	Price
Local contact	23236	Coomassie Plus - The Better Bradford Assay Kit <i>Sufficient reagents to perform 630 standard assays or 3,160 microplate assays.</i>	Kit		Local contact

Microbe Inotech Laboratories, Inc.
Summary Report of Analysis
[MILB – 3738A]

Joseph E. Dixon
 Danisco
 10994 Three Mile Road
 Thomson, IL 61285
 Phone: 815-259-3311
 Fax: 815-259-3611
 Email: joseph.dixon@danisco.com

February 17, 2006

Description and Chain of Custody Record Information:

- Tuesday February 7, 2006 – 9:08 AM: Received by courier, 4 solid samples for total protein determination performed by the Lowry-Folin method.
- Friday February 17, 2006 – 11:25 AM: Received notice to perform a total protein determination by the Bradford Method.
- MiL, Inc. REPORT & Invoice Number: MILB-3738A.
- Purchase Order: Credit Card.

Sample Processing:

Within 20 minutes of reception an aliquot from each sample is checked for weight or volume and serially diluted. The dilutions are aseptically transferred in a laminar flow biological cabinet and plated in the following manner(s):


- The Bradford Method has been found to be a most reliable and satisfactory method for quantization of soluble proteins. The procedure found in the attached pages is based on and utilizes Brilliant Blue G (Coomassie) that forms a stable complex with basic amino acid residues of proteins, mainly with arginine and aromatic amino acids.

Total Protein Determination


Total Protein Determination analysis results by the Bradford Method reported as milligram protein per gram of solid sample (mg/g)		
Sample	Test Type	Test Results
L120T5M18	Total Protein	0.5418 mg/g
N120T5C09A	Total Protein	0.5698 mg/g
N120T5L22	Total Protein	0.3246 mg/g
L120T5J06	Total Protein	0.2580 mg/g

See subsequent pages for data and protocol.

Thank you from the staff on project:



Bruce C. Hemming Ph.D.
President & CEO



Andrew William Johnson
Laboratory Manager

Bradford Protein Assay

Say:

Quantitative Endpoint assay with Standards and Unknowns (with and without dilution factor).

Principle:

When dissolved in an acid-alcoholic medium, the Brilliant Blue G dye reacts almost immediately with protein to form a blue-colored protein dye complex. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm. The amount of absorption at 595 nm is proportional to the protein present.

3705A												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.217	0.110	0.053	0.034	0.019	0.004						
B	0.224	0.101	0.061	0.028	0.021	-0.003						
C	0.049		0.027		0.034		0.057					
D	0.042		0.013		0.022		0.034					
E	0.044		0.004		0.039		0.001					
F	0.035		0.134		0.013		-0.003					
G												
H												

Endpoint

Lm1 600

Automix: Once
Calibrate: On

Plate Last Read:
3:15 PM 2/17/2006

Wavelength Combination: !Lm1

Data Mode: Absorbance

Plate Blank Used Lm1 = 0.521

Standards (µg/ml)

Sample	Concentration	Back Calc Conc	Wells	OD Values	Mean OD Value	Std.Dev.	CV%
St01	300.000	298.682	A1	0.217	0.220	0.005	2.2
		308.490	B1	0.224			
St02	150.000	148.757	A2	0.110	0.105	0.006	6.1
		136.147	B2	0.101			
St03	75.000	68.891	A3	0.053	0.057	0.006	10.0
		80.100	B3	0.061			
St04	37.500	42.268	A4	0.034	0.031	0.004	13.9
		33.861	B4	0.028			
St05	18.750	21.251	A5	0.019	0.020	0.001	7.3
		24.053	B5	0.021			

Unknowns (ug/ml)

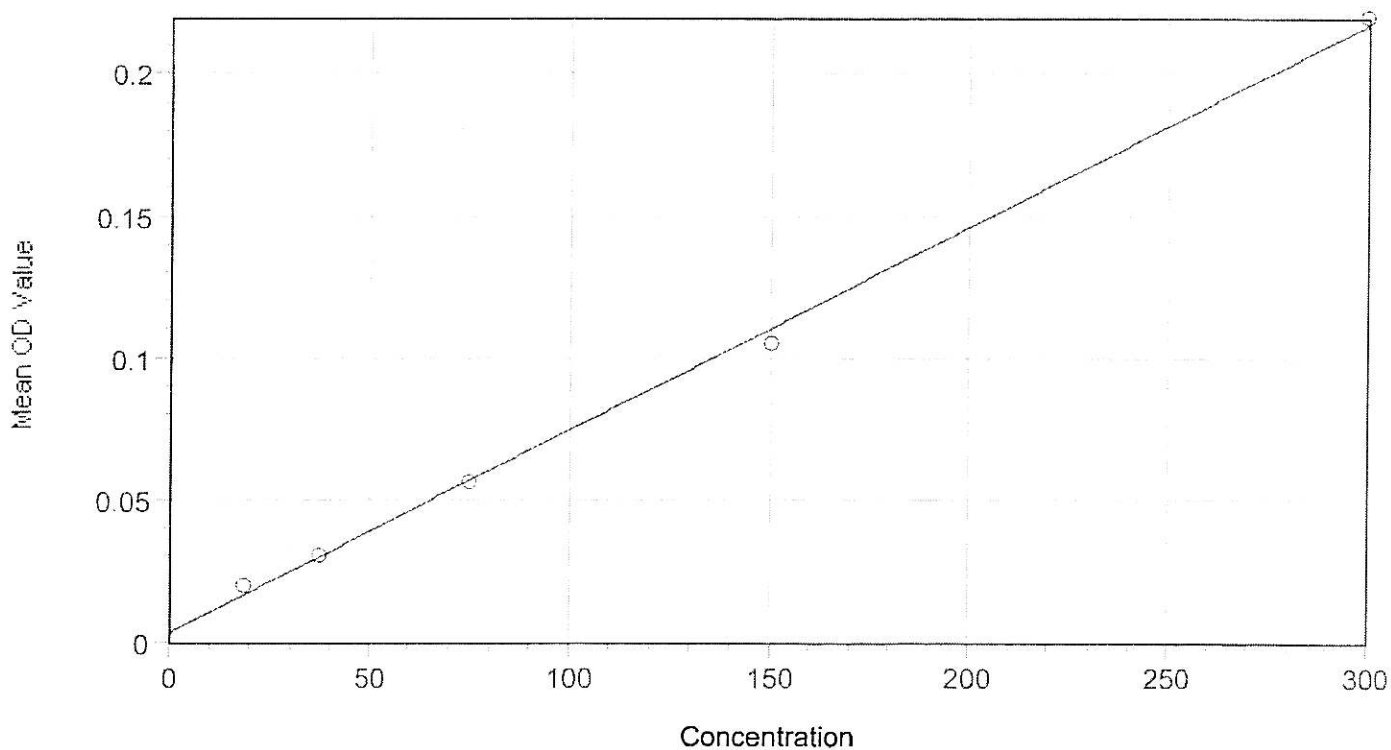
Sample	Wells	OD Values	Concentration	Mean Conc.	Std.Dev.	CV%	Dilution	Adj. Conc.
3705A-1	C1	0.049	63.286	54.178	8.130	15.0	10.0	541.784
	D1	0.042	53.478					
	E1	0.044	56.280					
	F1	0.035	43.670					
3705A-2	C3	0.027	32.460	56.981	84.648	148.6	10.0	569.807
	D3	0.013	12.844					
	E3	0.004	0.234					
	F3	0.134	182.385					
3705A-3	C5	0.034	42.268	32.460	16.460	50.7	10.0	324.603
	D5	0.022	25.454					
	E5	0.039	49.274					
	F5	0.013	12.844					
3705A-4	C7	0.057	74.495	25.805	39.917	154.7	10.0	258.048
	D7	0.034	42.268					
	E7	0.001	-3.970					
	F7	-0.003	-9.575					

Unknowns(no diln)

Sample	Wells	OD Values	R	Concentration	MeanConc	Std.Dev.	CV%
--------	-------	-----------	---	---------------	----------	----------	-----

R - Outside standard range

Standard Curve



$$y = A + Bx:$$

A

B

R²

STD#1 (Standards: Concentration vs Mean OD Va...

0.003

7.14e-4

0.998

29

Attachment 4

LOWRY PROTEIN ASSAY

P.J. Hansen

Dept. of Animal Sciences, University of Florida[More Techniques](#) | [P.J. Hansen Home Page](#) | [UF Animal Sciences Home Page](#)

The Lowry procedure is one of the most venerable and widely-used protein assays, being first described in 1951 [Lowry et al., *J. Biol. Chem.* 193: 265-275 (1951)]. Under alkaline conditions, copper complexes with protein. When folin phenol reagent (phospho-molybdic-phosphotungstic reagent) is added, the Folin-phenol reagent binds to the protein. Bound reagent is slowly reduced and changes color from yellow to blue.

While widely used, the Lowry procedure is less preferable an assay than some other protein assays since it is more subject to interference by a wide variety of chemicals. Among the chemicals reported to interfere with the Lowry procedure are barbital, CAPS, cesium chloride, citrate, cysteine, diethanolamine, dithiothreitol, EDTA, EGTA, HEPES, mercaptoethanol, Nonidet P-40, phenol, polyvinyl pyrrolidone, sodium deoxycholate, sodium salicylate, thimerosol, Tricine, TRIS and Triton X-100.

There is also much protein-to-protein variation in the intensity of color development. Ideally, the standard should be similar to the unknown. For example, if one is measuring IgG concentrations, an immunoglobulin standard would be ideal. For serum, use bovine serum albumin as a standard since albumin is a major component of serum.

An easy and accurate alternative, based on the binding of protein to Coomassie Blue G-250 dye, is the Bradford procedure. In addition, a modification of the Lowry procedure exists based on use of bicinchoninic acid (BCA) in place of the Folin-phenol reagent [Smith et al., *Anal Biochem.* 150, 76-85 (1985)]. The BCA is less prone to interference than the Lowry procedure and is more sensitive.

Stock Solutions

Lowry A: 2% Na_2CO_3 in 0.1 M NaOHLowry B: 1% CuSO_4 in diH_2O Lowry C: 2% sodium potassium tartrate ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$)

Reagents

Lowry stock reagent

49 ml Lowry A

0.5 ml Lowry B

0.5 ml Lowry C

Folin's Reagent: Phenol reagent - 2N (Folin - Ciocalteu reagent). Dilute 1:1 in diH_2O before use.

Standard: Bovine serum albumin (BSA), lysozyme or other protein. If the standard is weighed out before use, make sure that the protein preparation used to prepare the standard is salt-free to avoid inaccurate results. The standard should be dissolved at a concentration of 1 mg/ml in a buffer similar to the solution the unknown is in (use PBS for biological samples) and diluted as follows:

micrograms protein standard	microliters standard	microliters buffer
0	0	100
10	10	90
20	20	80
30	30	70
50	50	50
75	75	25
100	100	0

Procedure

- 1) Add 100 μ l of sample (sample + buffer = 100 μ l) per tube.
- 2) Add 1.0 ml of Lowry stock reagent to each tube.
- 3) Incubate 30 min at room temperature.
- 4) Add 100 μ l of Folin's reagent to each tube.
- 5) Incubate 30 min at room temperature.
- 6) Read in a spectrophotometer at 595 nm.

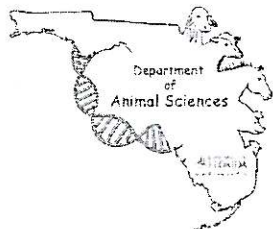
produced 7-90, html prepared 02-21-01. For questions, contact Peter J. Hansen



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FLORIDA Institute of Food and
Agricultural Sciences

Department of Animal Sciences - Dairy Program

Microbe Inotech Laboratories, Inc.
Summary Report of Analysis
[MILB – 3705A]

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 Thomson, IL 61285
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February 8, 2006
 Corrected 2.17.06

Description and Chain of Custody Record Information:

- Tuesday February 7, 2006 – 9:08 AM: Received by courier, 4 solid samples for total protein determination performed by the Lowry-Folin method.
- MiL, Inc. REPORT & Invoice Number: MILB-3705A.
- Purchase Order: 45090815

Sample Processing:

Within 20 minutes of reception an aliquot from each sample is checked for weight or volume and serially diluted. The dilutions are aseptically transferred in a laminar flow biological cabinet and plated in the following manner(s):

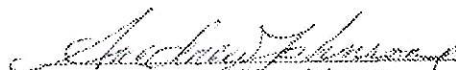
- The Lowry Method has been found to be the most reliable and satisfactory method for quantization of soluble proteins. The procedure found in the attached pages is based on Peterson's modification of the micro-Lowry method and utilizes sodium dodecylsulfate, included in the Lowry Reagent, to facilitate the dissolution of relatively insoluble lipoproteins.

Total Protein Determination

Total Protein Determination analysis results by the Lowry-Peterson Modification Method reported as milligram protein per gram of solid sample (mg/g)		
Sample	Test Type	Test Results
L120T5M18	Total Protein	0.5424 mg/g
N120T5C09A	Total Protein	0.4158 mg/g
N120T5L22	Total Protein	0.4120 mg/g
L120T5J06	Total Protein	0.4874 mg/g

See subsequent pages for data and protocol.

Thank you from the staff on project:


Bruce C. Hemming Ph.D.
President & CEO
Andrew William Johnson
Laboratory Manager

Protein Determination

Protein Assay

The Lowry procedure has been found to be the most reliable and satisfactory method for quantitation of soluble proteins. The procedure described here is based on Peterson's modification of the micro-Lowry method and utilized sodium dodecylsulfate, included in the Lowry Reagent, to facilitate the dissolution of relatively insoluble lipoproteins.

For many proteins, the Lowry reaction can be run directly on the protein solution. However, interference in the direct Lowry procedure is caused by commonly used chemicals, such as tris, ammonium sulfate, EDTA, sucrose citrate, amino acid and peptide buffers and phenols. The procedure with protein precipitation, which uses DOC (deoxycholate) and TCA (trichloroacetic acid), eliminates all these interferences with the exception of phenols. However, the amount of various proteins recovered through the precipitation step may vary depending on the particular protein assayed.

Assay:

Quantitative Endpoint assay with Standards and Unknowns (with and without dilution factor). The unknowns are interpolated from a standard curve.

Principle:

An alkaline cupric tartrate reagent complexes with the peptide bonds and forms a purple-color when the phenol reagent is added. Absorbance is read at a suitable wavelength between 500 nm and 800 nm. The protein concentration is determined from a calibration curve.

Danisco

MILB# 3705A

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.000	0.232	0.350	0.609	0.830	0.881							Endpoint
	0.000	0.232	0.350	0.609	0.830	0.881							Lm1 650
B	0.281	0.240	0.317	0.265									Automix: Off Calibrate: On
	0.281	0.240	0.317	0.265									
C	0.239	0.260	0.247	0.258									Plate Last Read: 2:57 PM 2/8/2006
	0.239	0.260	0.247	0.258									
D	0.237	0.255	0.254	0.255									
	0.237	0.255	0.254	0.255									
E	0.274	0.262	0.242	0.282									
	0.274	0.262	0.242	0.282									
F													
G													
H													

Wavelength Combination: !Lm1

Data Mode: Absorbance

Plate Blank Used Lm1 = 0.131

Standards (µg/ml)

Sample	Concentration	Wells	BackConcCalc	Values	MeanValue	Std Dev.	CV%
St01	50.000	A2	31.858	0.232	0.232	0.000	0.0
St02	100.000	A3	92.200	0.350	0.350	0.000	0.0
St03	200.000	A4	224.623	0.609	0.609	0.000	0.0
St04	300.000	A5	337.617	0.830	0.830	0.000	0.0
St05	400.000	A6	363.692	0.881	0.881	0.000	0.0

Smallest standard value: 0.232

Largest standard value: 0.881

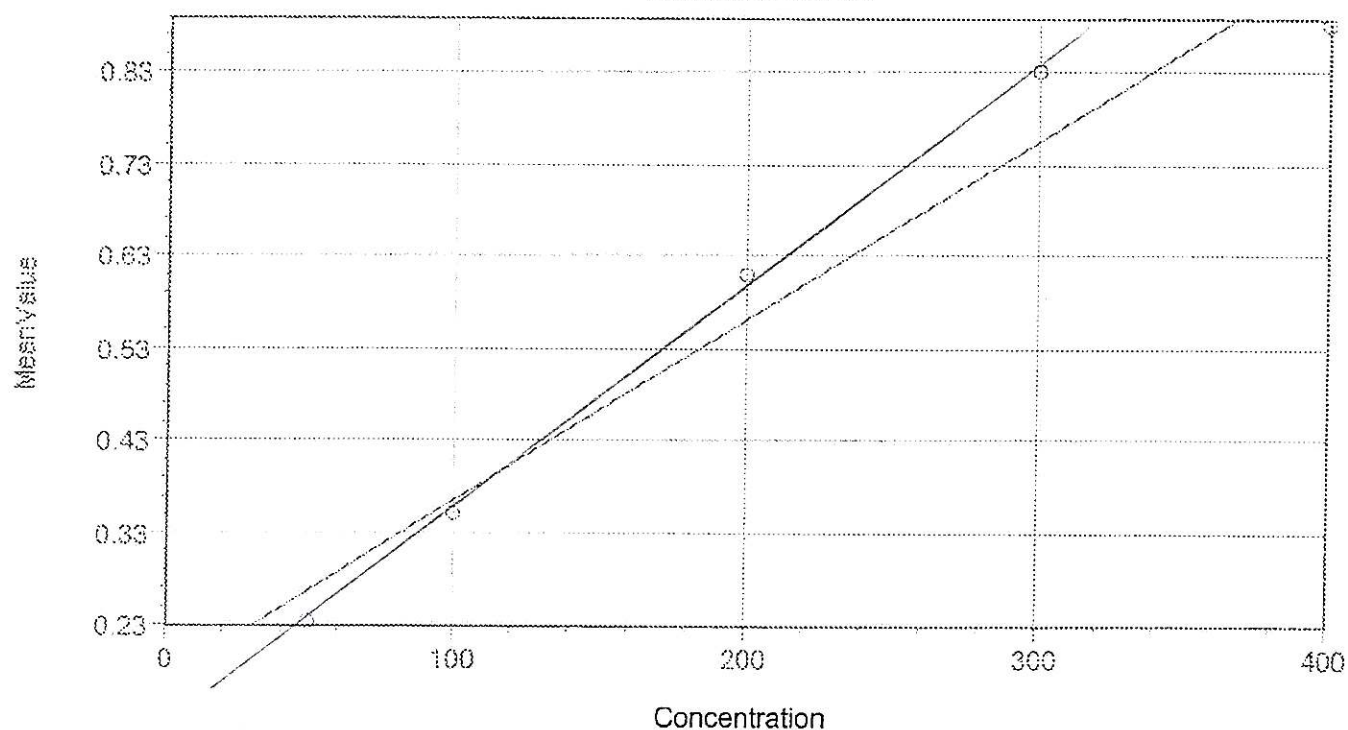
20

Samples

Sample	Wells	Values	Concentration	MeanValue
L120T5J06	E1	0.274	53.342	48.740
	E2	0.262	47.207	
	E3	0.242	36.981	
	E4	0.282	57.432	
L120T5M18	B1	0.281	56.921	54.237
	B2	0.240	35.958	
	B3	0.317	75.327	
	B4	0.265	48.740	
N120T5C09A	C1	0.239	35.447	41.582
	C2	0.260	46.184	
	C3	0.247	39.537	
	C4	0.258	45.161	
N120T5L22	D1	0.237	34.424	41.199
	D2	0.255	43.628	
	D3	0.254	43.116	
	D4	0.255	43.628	

Outlier - Outside standard range

Standard Curve



$$y = A + Bx:$$

A

B

R²

○ Std (Standards: Concentration vs MeanValue)

0.17

0.002

0.957

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Veratox for Total Milk Allergen is used for the quantitative analysis of milk residue in food products such as juices, wine, sauces or sorbets.

The Test

The test is a sandwich ELISA that provides exact concentrations in parts per million (ppm). Any present milk residue is extracted from samples with a buffered salt solution (PBS) by shaking in a heated water bath. Extracted milk residue is sampled and added to antibody-coated wells (capture antibody). Any unbound residue is washed away and a second, enzyme-labeled antibody (detector antibody) is added. The detector antibody binds to the already bound milk residue. After a second wash, the substrate is added. Color develops as a result of the presence of bound detector antibody. Red Stop reagent is added and the color of the resulting solution is observed. A microwell reader is used to yield optical densities. Control optical densities form a standard curve, and sample optical densities are plotted against the curve to calculate the exact concentration of milk residue.

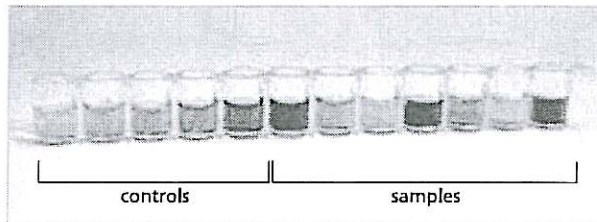
The Procedure

Samples must be extracted prior to testing.

1. Add 150 μ L controls and extracted samples to transfer wells.
2. Transfer 100 μ L to the antibody wells. Incubate for 10 minutes.
3. Dump liquid from antibody wells.
4. Wash wells thoroughly with wash buffer. Tap out water on paper towel.
5. Transfer 100 μ L conjugate from reagent boat to antibody wells using 12-channel pipettor. Incubate for 10 minutes.
7. Repeat steps 3-4 by dumping out the liquid, thoroughly washing the wells, and tapping dry.
8. Transfer 100 μ L substrate from reagent boat to antibody wells using 12-channel pipettor. Incubate for 10 minutes.
9. Transfer 100 μ L Red Stop from reagent boat to antibody wells.
10. Read results in a microwell reader.

See package insert for complete instructions

Results



Product Specifications

Range of quantitation:	2.5 ppm - 25 ppm
Controls provided:	0, 2.5, 5, 10 and 25 ppm non-fat dry milk
Testing time:	30 minutes
Tests per kit:	Up to 38

Materials Recommended But Not Provided

Available from Neogen*

1. Allergen Extraction Kit
2. Adjustable 50-200 μ L pipettor
3. Graduated cylinder capable of measuring 125 mL
4. Scale capable of weighing 5 ± 0.1 g
5. Microwell reader with a 650 nm filter
6. 50-200 μ L pipettor
7. 12-channel pipettor
8. Tips for adjustable and 12-channel pipettors
9. Timer
10. Microwell holder
11. Wash bottle
12. 3 reagent boats for use with 12-channel pipettor
13. 1.0 L bottle to prepare washing solution
14. 1.0 L heat safe bottle to prepare extract solution

Not available from Neogen

1. Shaker water bath adjusted to 60°C (140°F) with clamps to hold extraction bottles
2. Paper towels or equivalent absorbent material
3. Waterproof marker
4. Distilled or deionized water

* See *Equipment and Accessories*, pages 91-95

Ordering Information

Prod.#	Product description
8470	Veratox for Total Milk Allergen
8429	Allergen Extraction Kit - 20 samples

Close Window 

Allergens, Milk

The milk allergen test is a sandwich enzyme-linked immunoassay (ELISA). Typical samples applicable to the ELISA are sauces, juices, sorbets and environmental surfaces.

Milk protein is extracted from samples with a buffered salt solution by shaking in a heated water bath followed by filtering or centrifuging. Extracted milk protein is added to anti-body coated wells where it binds to the antibody over time. The well is rinsed followed by addition of a second enzyme labeled antibody which binds to the already bound protein, if present. This well is rinsed again followed by the addition of a substrate. A color reaction takes place over time followed by a stop reagent. Blue color signifies allergen present, red color indicates little, < 2.5 ppm, or no milk present.

DETECTION RANGE: 2.5-15 ppm.




MISCELLANEOUS INFORMATION: Please try to submit good representative samples, frozen and at least 100 gram quantities.


Neofen

Pricing Information

\$100 per analysis

Download Forms

-  Nutritional Request
-  Analytical Request
-  Shipping Instructions

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MEDALLION LABS

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Final Report

Report Date: December 29, 2005
 Date Submitted: December 22, 2005
 Company Code: DANISCO07

Micheal Auerbach
 Danisco USA
 10994 Three Mile Road
 Thompson, IL 61285

Library Number: **2005-11259**
 PO Number: 45089262

Medallion Labs Sample ID: 2005087442

Lectitol Monohydrate

Customer Sample ID: L120T5J06

Micheal Auerbach, Danisco USA

Assay	Component	Results	Units
Dairy Allergen Kit, ELISA	Dairy Allergens	<2.5	mg/kg (ppm)

* * * * *

Medallion Labs Sample ID: 2005087443

Lectitol Monohydrate

Customer Sample ID: L120T5M18

Micheal Auerbach, Danisco USA

Assay	Component	Results	Units
Dairy Allergen Kit, ELISA	Dairy Allergens	<2.5	mg/kg (ppm)

* * * * *

Medallion Labs Sample ID: 2005087444

Anhydrous Lactitol

Customer Sample ID: N120T5C09A

Micheal Auerbach, Danisco USA

Assay	Component	Results	Units
Dairy Allergen Kit, ELISA	Dairy Allergens	<2.5	mg/kg (ppm)

* * * * *

Medallion Labs Sample ID: 2005087445

Anhydrous Lactitol

Customer Sample ID: N120T5L22

Micheal Auerbach, Danisco USA

Assay	Component	Results	Units
Dairy Allergen Kit, ELISA	Dairy Allergens	<2.5	mg/kg (ppm)

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